Letter to the Editor

Eloísa Urrechaga*, Urko Aguirre, Pablo Pedro España and Luis García de Guadiana

Complete blood counts and cell population data from Sysmex XN analyser in the detection of SARS-CoV-2 infection

https://doi.org/10.1515/cclm-2020-1416
Received July 17, 2020; accepted October 11, 2020;
published online October 20, 2020

Keywords: complete blood counts; cell population data; COVID-19; Sysmex XN.

To the Editor,

The first cases of coronavirus disease 2019 (COVID-19) probably occurred in Wuhan (China) in December 2019. The first case in Spain was confirmed on January 31st, 2020 and in our region (Basque Country), on February 28th [1].

Recently, Lippi et al. [2, 3] have reviewed the role of clinical tests in the handling of SARS-CoV-2 pandemic.

Some of the features of the complete blood count (CBC) and the leucocyte differential in COVID-19 patients might be useful for COVID screening and prognosis. Conditions such as neutrophilia, lymphopenia and thrombocytopaenia and changes in the levels of markers of systemic inflammation (e.g., neutrophil-to-lymphocyte ratio, NLR), are the most significant quantitative alterations. They have been recognised as valid predictors of disease severity [4, 5]. Morphological anomalies of circulating blood cells have also been reported in COVID-19 [6].

The XN analyser (Sysmex, Kobe, Japan) can supply can supply detailed information for the necessary morphological studies of leucocytes and the assessment of alterations triggered by infections. The following cell population data (CPD), obtained using this analyser, are related to the morphology and functional characteristics of leucocytes:

- internal cellular complexity: NE-X, LY-X, MO-X, NE-WX, LY-WX, and MO-WX
- nucleic acid content: NE-Y, LY-Y, MO-Y, NE-WY, LY-W and MO-WY
- cell size: NE-Z, LY-Z, MO-Z, NE-WZ, LY-WZ and MO-WZ

In recent years, the studies of CPD have shown changes in the morphology of activated leucocytes in acute infections and sepsis [7, 8]. We examined the CBC and CPD data of the patients infected with SARS-CoV-2 virus and other infections of different aetiologies. We assessed these parameters as early laboratory indicators for the detection of COVID-19.

This prospective observational study was conducted after receiving approval from the Hospital Research Ethical Committee.

The study group consisted of consecutive patients with fever admitted to the Emergency Room at Galdakao–Usansolo Hospital in the period between 30th of March and 10th of April 2020. The criterion for inclusion in the validation group was the same as for the study group (consecutive patients with fever admitted to the Emergency Room between 15th and 30th of April).

Patients in life-threatening clinical condition were excluded from the study.

The diagnoses of each patient was retrieved from their laboratory and medical records. We obtained the following data: age, gender, clinical symptoms, underlying diseases, and the initial (admission) laboratory findings.

The CBC and CPD were acquired in the Emergency Laboratory, using the XN20 analyser. The counter was calibrated, monitored and maintained following the manufacturer's recommendations. The long-term consistency for CPD parameters was routinely assessed using the so-called moving average; the overall quality assessment has been described elsewhere [8].

***Corresponding author: Eloísa Urrechaga**, Biocruces Bizkaia Health Research Institute, Hospital Galdakao – Usansolo, 48960 Galdakao, Baracaldo, Vizcaya, Spain, Phone: +34 94 400 7000,
E-mail: eloisa.urrechagaigartua@osakidetza.net
Urko Aguirre, Research Network in Health Services in Chronic Diseases (Red de Investigación en Servicios de Salud en Enfermedades Crónicas, REDISSEC), Research Unit of the Barrualde-Galdakao IHO, Galdakao, Spain
Pablo Pedro España, Department of Pneumology, Hospital Galdakao, Usansolo, Spain
Luis García de Guadiana, Medicine Laboratory Department, University Hospital Santa Lucía, Cartagena, Spain

Patients with COVID-19 were diagnosed following the current standards, and on the basis of positive results of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2. Bacterial infections were revealed by positive cultures, while positive serology or molecular testing documented viral infections.

The statistical analysis included a preliminary data analysis, using medians and interquartile range (IQR) for continuous variables and frequencies and percentages for categorical data. The Mann–Whitney test was applied to detect statistical differences between groups. To test the hypothesis that the patients with infection/fever could be categorised into distinct groups based on the aetiology of the infection by evaluating all the analytical data, the unsupervised K-means clustering method was applied. To obtain the optimal number of clusters, a set of indices, including standard and new research laboratory tests (CBC, CPD), was computed, varying all the combinations in cluster number. Moreover, the principal component analysis (PCA) was used to validate the choice of the optimal number of clusters, and to plot the data points according to the optimal PCA obtained. Before the clustering, all the studied CPD parameters had been scaled, normalised and converted to z-scores.

Differences between CPD values were compared employing the non-parametric Wilcoxon test. The diagnostic performance of individual parameters in the differential diagnosis of infections with diverse aetiologies was evaluated using receiver operating characteristic curve analysis. A p-value <0.05 was considered statistically significant. All the statistical procedures were performed using SAS System 9.4 and R v. 4.0 release.

In the study group, 72 patients suffered from pneumonia, urinary tract infections, meningitis and gastroenteritis caused by bacteria and 45 viral infections (respiratory infections, Epstein-Barr virus, influenza). COVID-19 was confirmed in 153 patients, 100 males with a mean age of 63.2 years (range 29–94 years) and 53 women with a mean age of 69.2 years (range 38–94 years).

The validation group consisted of 92 patients, of whom 43 were diagnosed with COVID-19, and 49 suffered other

Table 1: Median and 25–75 percentile range (P25, P75) data for leucocytes (absolute counts, 10^9/L) and cell population data (arbitrary optical units) in the study and validation groups.

	Study group				Validation group	
	COVID-19 (n=153)	Bacterial (n=72)	Viral (n=45)	p-value	COVID-19 (n=43)	Non-COVID-19 (n=49)
WBC	7.15 (5.32, 9.57)	17.5 (15.91, 20.53)	7.14 (6.23, 8.62)	0.053	7.21 (5.66, 9.07)	15.56 (14.72.18.22)
NLR	5.17 (3.11, 8.39)	9.98 (5.86, 17.22)	1.18 (0.76, 2.10)	0.01	6.01 (4.11, 7.55)	10.5 (4.99.15.5)
Neut	6.91 (4.52.12.2)	14.71 (12.9.17.31)	3.66 (2.33. 4.44)	0.05	6.1 (5.21, 11.2)	12.83 (11.8, 15.33)
Lymph	1.01 (0.72, 1.50)	1.57 (0.98, 4.49)	2.39 (1.66, 4.24)	0.05	1.11 (0.77, 1.33)	1.35 (1.1.4.04)
NE X	155 (152, 159)	150 (147, 154)	154 (150, 157)	0.28	153 (153, 157)	152 (150,154)
NE Y	50 (48, 51)	52 (49, 54)	50 (48, 51)	0.30	51 (49, 51)	52 (50.53)
NE Z	85 (82, 88)	86 (83, 88)	90 (86, 93)	0.52	87 (84, 88)	88 (84.88)
NE-WX	314 (302, 325)	315 (307, 330)	313 (303, 325)	0.345	316 (310, 323)	315 (309, 325)
NE-WY	630 (605, 669)	654 (617, 688)	589 (571, 608)	0.25	628 (605, 651)	648 (624,658)
NE-WZ	641 (612, 668)	776 (738, 816)	762 (738, 798)	0.44	647 (615, 661)	770 (744,781)
LY X	82 (80, 83)	80 (78, 82)	83 (81, 84)	0.69	82 (80, 82)	80 (78.81)
LY Y	71 (68, 73)	71 (67, 75)	70 (68, 72)	0.69	72 (70, 73)	71 (68, 72)
LY Z	55 (55, 56)	58 (57, 60)	61 (60, 62)	0.63	56 (55, 56)	60 (58.62)
LY-WX	500 (463, 543)	482 (446, 522)	466 (434, 516)	0.14	508 (471, 528)	486 (463,523)
LY-WY	900 (864, 990)	896 (845, 1026)	891 (816, 1019)	0.04	920 (883, 963)	892 (816,999)
LY-WZ	519 (492, 554)	660 (624, 695)	654 (621, 710)	0.41	515 (491, 543)	655 (633,689)
MO X	125 (123, 126)	120 (118, 122)	124 (122, 125)	0.53	124 (123, 125)	121 (118,124)
MO Y	117 (110, 123)	114 (110, 121)	115 (109, 120)	0.61	116 (110, 120)	115 (112,120)
MOZ	62 (60, 64)	68 (67, 69)	71 (69, 74)	0.08	64 (60, 64)	68 (67.69)
MO-WX	253 (237, 268)	261 (244, 277)	239 (225, 254)	0.52	256 (239, 267)	263 (254,273)
MO-WY	694 (616, 774)	719 (673, 806)	676 (606, 731)	0.14	688 (636, 763)	706 (668,753)
MO-WZ	583 (529, 624)	721 (674, 784)	736 (679, 817)	0.14	593 (540, 622)	727 (683,779)

WBC, leucocyte count, 10^9/L ; Neut, neutrophils, 10^9/L; Lymph, lymphocytes, 10^9/L; NLR, neutrophil/lymphocyte ratio; NE-X, neutrophil complexity; NE-Y, neutrophil fluorescence; NE-Z, neutrophil size; NE-WX, width of dispersion of neutrophil complexity; NE-WY, width of dispersion of neutrophil fluorescence; NE-WZ, width of dispersion of neutrophil size; LY-X, lymphocyte complexity; LY-Y, lymphocyte fluorescence; LY-Z, lymphocyte size; LY-WX, width of dispersion of lymphocyte complexity; LY-WY, width of dispersion of lymphocyte fluorescence; LY-WZ, width of dispersion of lymphocyte size; MO-X, monocyte complexity; MO-Y, monocyte fluorescence; MO-Z, monocyte size; MO-WX, width of dispersion of monocyte complexity; MO-WY, width of dispersion of monocyte fluorescence; MO-WZ, width of dispersion of monocyte size.

Table 2: Comparison of the diagnoses in the validation group, COVID-19 (n=43) and non-COVID-19 (n=49), with the predicted classification, applying K-means technique; 42 out of 43 COVID-19 patients were correctly classified into the corresponding cluster.

Validation group		Clusters		True +	True−	False +	False−
	n	COVID-19	Non-COVID-19				
COVID-19	43	42 (97.7)	1 (2.3)	42			1
Non-COVID-19	49	15 (30.6)	34 (69.4)		34	15	
Total	92	57	35				

infections (44 bacterial five viral). Their analytical data are summarised in Table 1.

The NLR was very useful in distinguishing the viral infections from bacterial and SARS-CoV-2 infections, the last two characterised by neutrophilia. The area under the curve was 0.939 (95% confidence interval 0.900–0.965). At a cut-off ≤3.31, sensitivity was 100% and specificity 77.2% to rule out viral non-COVID-19 infections.

The NLRs were significantly different in COVID-19 and bacterial infections (medians 5.17 and 9.98, respectively), but the values in the two groups overlapped.

The mathematical method of clustering performed well in discriminating the COVID 19 patients: 143 out of 153 COVID-19 patients (93.5%) and 100% of non-COVID-19 patients were correctly classified.

This method was then applied to the validation group, and the correct classification was obtained for 97.7% of COVID-19 and 70% of non-COVID-19 infections (100% of viral and 70% of bacterial infections). For the COVID-19 detection, the positive predictive value was 74.1% and negative predictive value, 97.1% (Table 2).

We can hypothesize that the morphological alterations in leucocytes, quantified by using CPD values, represent transformations in the functionality of these cells taking place during the SARS-CoV-2 infection. However, the fact that the CPD are research parameters makes it difficult to manage the information they provide.

Several studies have reported that the NLR values increase significantly in patients with severe COVID-19; it might be considered an independent biomarker to indicate poor clinical outcomes [9]. The present study highlights its potential value in ruling out non-COVID-19 viral infections, with high diagnostic performance. The clustering and PCA applied to the CPD values are also potent methods for distinguishing the COVID-19 patients from others (97.7% were correctly classified).

The results look promising they show a high rate of correct classification of patients suffering from infections of different aetiologies.

Our study had some limitations: first, it was conducted in only one hospital; the results must be validated employing a multicentre evaluation, including more patients. Second, it is not yet clear how such useful CPD information might be reported to the clinicians effectively. We suggest a flag of "suspected COVID-19", based on the NLR and CPD values.

During the COVID-19 outbreak, the emergency departments have become the first line of defence in the hospitals; they are often the departments receiving and screening the suspected cases. The CBC is the laboratory test most frequently ordered by emergency physicians; its results, in combination with CPD data, could assist in the initial evaluation of patients with fever of unknown origin. It should also help in reducing the threat of nosocomial infections of nurses and medical staff.

Acknowledgments: In memory of our colleague Mrs. E. V. V., a nurse at the Pneumatology Ward of Galdakao–Usansolo Hospital, who died of COVID-19 on the 18th of March 2020.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This prospective observational study was conducted after receiving approval from the Hospital Research Ethical Committee.

References

1. Centro de Coordinación de Alertas y Emergencias Sanitarias. Enfermedad por el coronavirus (COVID-19). España: Ministerio de Sanidad. Available from: https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov-China/situacionActual.htm.
2. Lippi G, Plebani M. The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. Clin Chem Lab Med 2020;58:1063–9.

3. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med 2020;58:1131–4.
4. Frater JL, Zini G, d'Onofrio G, Rogers HJ. COVID-19 and the clinical hematology laboratory. Int J Lab Hematol 2020;42:11–8.
5. Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGE, Tan GB, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol 2020;95:E131–4.
6. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. Am J Hematol 2020;95: 870–2.
7. Urrechaga E, Bóveda O, Aguirre U. Role of leucocytes cell population data in the early detection of sepsis. J Clin Pathol 2018;71:259–66.
8. Urrechaga E, Bóveda O, Aguirre U. Improvement in detecting sepsis using leukocyte cell population data. Clin Chem Lab Med 2019;57: 918–26.
9. Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. J Med Virol https://doi.org/10.1002/jmv.25819.